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# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS Geneva

# **TECHNICAL COMMITTEE**

# Fifty-Second Session Geneva, March 14 to 16, 2016

REVISION OF DOCUMENT TGP/8: PART I: DUS TRIAL DESIGN AND DATA ANALYSIS, NEW SECTION: MINIMIZING THE VARIATION DUE TO DIFFERENT OBSERVERS OF THE SAME TRIAL

#### Document prepared by the Office of the Union

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# **EXECUTIVE SUMMARY**

1. The purpose of this document is to present a draft of a new section for document TGP/8 Part I: DUS Trial and Design and Data Analysis, on "Minimizing the Variation due to Different Observers of the Same Trial".

# 2. The TC is invited to:

- (a) consider the draft guidance on "Minimizing the variation due to different observers of the same trial", as presented in the Annex to this document, in conjunction with the comments by the TWPs at their sessions in 2015, and the TC-EDC at its meeting in 2016, as a basis for a future revision of document TGP/8: "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability", Part I: DUS Trial Design and Data Analysis;
- (b) consider whether to develop another section in the document to provide guidance on minimizing the variation due to different observers of the same trial for PQ characteristics, including the use of non-parametric methods;
- (c) consider whether to request the TWPs at their sessions in 2016 to provide information on variation between observers for PQ characteristics, such as frequency of deviations;
- (d) note that the TWC had agreed to invite the experts from Argentina and Brazil to make a presentation at its thirty fourth session on their experiences in training for minimizing variation between observers on PQ characteristics:
- (e) consider whether to invite experts to present to the TWPs, at their sessions in 2016, their experiences with regard to minimizing the variation due to different observers of the same trial for PQ characteristics.

3. The structure of this document is as follows:

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4. The following abbreviations are used in this document:

CAJ: Administrative and Legal Committee

TC: Technical Committee

TC-EDC: Enlarged Editorial Committee

TWA: Technical Working Party for Agricultural Crops

TWV: Technical Working Party for Vegetables

TWC: Technical Working Party on Automation and Computer Programs

TWF: Technical Working Party for Fruit Crops

TWO: Technical Working Party for Ornamental Plants and Forest Trees

TWPs: Technical Working Parties

#### **BACKGROUND**

5. Document TGP/8/1 Draft 7 PART I, paragraph 2.9.1: "Control of variation due to different observers", considered by the Technical Working Parties, at their sessions in 2007, states:

"[If this section is required, TWPs are invited to contribute guidance on the control of variation due to different observers when statistical analysis is not used to determine distinctness and to consider it in relation to paragraph 2.7.2.9.]"

6. Developments prior to 2015 are presented in document TC/51/16 "Revision of document TGP/8: Part I: DUS Trial Design and Data Analysis, New Section: Minimizing the Variation due to Different Observers".

# **DEVELOPMENTS IN 2015**

# **Technical Committee**

- 7. The TC, at its fifty-first session, held in Geneva, from March 23 to 25, 2015, considered document TC/51/16 "Revision of document TGP/8: Part I: DUS Trial Design and Data Analysis, New Section: Minimizing the Variation due to Different Observers" and the draft guidance for inclusion in document TGP/8 on minimizing the variation due to different observers of the same trial, as reproduced in the Annex to document TC/51/16, in conjunction with the comments of the TWPs at their sessions in 2014 (see document TC/51/39, paragraph 132).
- 8. The TC agreed to request the expert from Australia to continue developing the document to be presented to the TWPs at their sessions in 2015, and to amend the title to that used in the Annex to document TC/51/16 "Minimizing the variation due to different observers of the same trial" (see document TC/51/39, paragraph 133).
- 9. In response to the request of the TC, the drafter from Australia (Mr. Nik Hulse), has provided revised draft guidance for future revision of document TGP/8 on minimizing the variation due to different observers, for consideration by the TWPs at their sessions in 2015, as presented in the Annex to this document.

#### **Technical Working Parties**

- 10. The TWV, TWC, TWA, TWF and TWO considered documents TWV/49/15, TWC/33/15, TWA/44/15, TWF/46/15 and TWO/48/15, respectively.
- 11. The TWV and TWC agreed that the draft guidance in the Annex to documents TWV/49/15 and TWC/33/15 should continue to be developed for inclusion in a future revision of document TGP/8 on minimizing the variation due to different observers.
- 12. The TWV suggested that further consideration should be given to guidance on PQ characteristics and proposed the development of another section in the document to explain non-parametric methods. The TWV also encouraged the other TWPs to consider whether further work should be done on PQ characteristics in the draft guidance.
- 13. The TWC agreed that further information should be provided on variation between observers for PQ characteristics before guidance could be drafted on the use of non-parametric methods, such as frequency of deviations.
- 14. The TWC agreed to invite the experts from Argentina and Brazil to make a presentation at its thirty-fourth session on their experiences in training for minimizing variation between observers on PQ characteristics.
- 15. The TWA agreed with the draft guidance in the Annex to document TWA/44/15, for inclusion in a future revision of document TGP/8 on minimizing the variation due to different observers.
- 16. The TWF and TWO agreed with the draft guidance in the Annex to document TWF/46/15, for inclusion in a future revision of document TGP/8 on minimizing the variation due to different observers, subject to the following editorial change:

"However, the method has not been used on developed for PQ characteristics to our knowledge and PQ characteristics—may also require extra information on calibration".

### **DEVELOPMENTS IN 2016**

## **Enlarged Editorial Committee**

17. The TC-EDC, at its meeting held in Geneva, on January 6 and 7, 2016, considered document TC-EDC/Jan16/5 "Revision of document TGP/8: Part I: DUS Trial Design and Data Analysis, New Section: Minimizing the Variation due to Different Observers of the Same Trial" and made the following proposals:

Annex, Par. 2.2	to read: "However, the way that a characteristic is observed or measured may vary per <a href="mailto:vear">vear</a> , location or testing authority. Calibration manuals made by the local testing authority <a href="mailto:and-example-varieties">and-example-varieties</a> are very useful for the local implementation of the UPOV Test Guidelines"
Annex, Par. 2.3	to read: "The Glossary of Terms Used in UPOV Documents (document TGP/14/2) provides useful guidance for clarifying many characteristics, in particular PQ characteristics.

# **PROPOSAL**

18. It is proposed to consider the draft guidance on "Minimizing the variation due to different observers of the same trial", as presented in the Annex to this document, in conjunction with the comments by the TWPs at their sessions in 2015, and the TC-EDC at its meeting in 2016, as a basis for a future revision of document TGP/8: "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability", Part I: DUS Trial Design and Data Analysis.

# 19. The TC is invited to:

- (a) consider the draft guidance on "Minimizing the variation due to different observers of the same trial", as presented in the Annex to this document, in conjunction with the comments by the TWPs at their sessions in 2015, and the TC-EDC at its meeting in 2016, as a basis for a future revision of document TGP/8: "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability", Part I: DUS Trial Design and Data Analysis, including in particular whether to develop another section in the document to provide guidance on minimizing the variation due to different observers of the same trial for PQ characteristics, including the use of non-parametric methods;
- (b) consider whether to request the TWPs at their sessions in 2016 to provide information on variation between observers for PQ characteristics, such as frequency of deviations;
- (c) note that the TWC agreed to invite the experts from Argentina and Brazil to make a presentation at its thirty-fourth session on their experiences in training for minimizing variation between observers on PQ characteristics;
- (d) consider whether to invite experts to present to the TWPs, at their sessions in 2016, their experiences with regard to minimizing the variation due to different observers of the same trial for PQ characteristics.

[Annex follows]

#### **ANNEX**

# TGP/8/1: PART I: NEW SECTION: MINIMIZING THE VARIATION DUE TO DIFFERENT OBSERVERS OF THE SAME TRIAL

Compared to the version presented to the TC at its fifty-first session, the proposed changes are presented below in highlight and underline (insertion) and strikethrough (deletion).

# 1. Introduction

This document considers variation between observers of the same trial at the authority level. It has been prepared with QN/MG, QN/MS, QN/VG and QN/VS characteristics in mind. It does not explicitly deal with PQ characteristics like color and shape. The described Kappa method in itself is largely applicable for these characteristics, e.g. the standard Kappa characteristic is developed for nominal data. However, the method has not been used on developed for PQ characteristics to our knowledge and PQ characteristics may also require extra information on calibration. As an example, for color calibration, you also have to take into account the RHS Colour chart, the lighting conditions and so on. Differences between observers on PQ characteristics could be tested using non-parametric methods, such as frequency of deviations. These aspects are not covered in this document.

- 1.1 Variation in measurements or observations can be caused by many different factors, like the type of crop, type of characteristic, year, location, trial design and management, method and observer. Especially for visually assessed characteristics (QN/VG or QN/VS) differences between observers can be the reason for large variation and potential bias in the observations. An observer might be less well trained, or have a different interpretation of the characteristic. So, if observer A assesses variety 1 and observer B variety 2, the difference observed might be caused by differences between observers A and B instead of differences between varieties 1 and 2. Clearly, our main interest lies with the differences between varieties and not with the differences between the observers. It is important to realize that the variation caused by different observers cannot be eliminated, but there are ways to control it.
- 1.2 It is recommended that, wherever possible, one observer should be used per trial to minimize variation in observations due to different observers.

#### 2. Training and importance of clear explanations of characteristics and method of observation

- 2.1 Training of new observers is essential for consistency and continuity of plant variety observations. Calibration manuals, supervision and guidance by experienced observers as well as the use of example varieties illustrating the range of expressions are useful ways to achieve this.
- 2.2 UPOV test guidelines try to harmonize the variety description process and describe as clearly as possible the characteristics of a crop and the states of expression. This is the first step in controlling variation and bias. However, the way that a characteristic is observed or measured may vary per <a href="year">year</a>, location or testing authority. Calibration manuals made by the local testing authority <a href="and example varieties">and example varieties</a> are very useful for the local implementation of the UPOV test guideline. Where needed these crop-specific manuals explain the characteristics to be observed in more detail, and specify when and how they should be observed. Furthermore they may contain pictures and drawings for each characteristic, often for every state of expression of a characteristic.
- 2.43 The Glossary of Terms Used in UPOV Documents (document TGP/14/2) provides useful guidance for clarifying many characteristics, in particular PQ characteristics.
- 2.34 Once an observer is trained it is important to ensure frequent refresher training and recalibration.

# 3. Testing the calibration

- 3.1 After training an observer, the next step could be to test the performance of the observers in a calibration experiment. This is especially useful for inexperienced observers who have to make visual observations (QN/VG and QN/VS characteristics). If making visual observations, they should preferably pass a calibration test prior to making observations in the trial. But also for experienced observers, it is useful to test themselves on a regular basis to verify if they still fulfill the calibration criteria.
- 3.2 A calibration experiment can be set up and analyzed in different ways. Generally it involves multiple observers, measuring the same set of material and assessing differences between the observers.

# 4. Testing the calibration for QN/MG or QN/MS characteristics

- 4.1 For observations made by measurement tools, like rulers (often QN/MS characteristics), the measurement is often made on an interval or ratio scale. In this case, the approach of Bland and Altman (1986) can be used. This approach starts with a plot of the scores for a pair of observers in a scatter plot, and compare it with the line of equality (where y=x). This helps the eye gauging the degree of agreement between measurements of the same object. In a next step, the difference per object is taken and a plot is constructed with on the y-axis the difference between the observers and on the x-axis either the index of the object, or the mean value of the object. By further drawing the horizontal lines y=0, y=mean(difference) and the two lines  $y=mean(difference) \pm 2 x$  standard deviation, the bias between the observers and any outliers can easily be spotted. Similarly we can also study the difference between the measurement of each observer and the average measurement over all observers. Test methods like the paired t-test can be applied to test for a significant deviation of the observer from another observer or from the mean of the other observers.
- 4.2 By taking two measurements by each observer of every object, we can look at the differences between these two measurements. If these differences are large in comparison to those for other observers, this observer might have a low repeatability. By counting for each observer the number of moderate and large outliers (e.g. larger than 2 times and 3 times the standard deviation respectively) we can construct a table of observer versus number of outliers, which can be used to decide if the observer fulfills quality assurance limits.
- 4.3 Other quality checks can be based on the repeatability and reproducibility tests for laboratories as described in ISO 5725-2. Free software is available on the ISTA website to obtain values and graphs according to this ISO standard.
- 4.4 In many cases of QN/MG or QN/MS, a good and clear instruction usually suffices and variation or bias in measurements between observers is often negligible. If there is reason for doubt, a calibration experiment as described above can help in providing insight in the situation.
- 4.5 In the case of QN/MG observations consideration and allowance may need to be given to the possible random within plot variation.

# 5. Testing the calibration for QN/VS or QN/VG characteristics

- 5.1 For the analysis of ordinal data (QN/VS or QN/VG characteristics), the construction of contingency tables between each pair of observers for the different scores is instructive. A test for a structural difference (bias) between two observers can be obtained by using the Wilcoxon Matched-Pairs test (often called Wilcoxon Signed-Ranks test).
- 5.2 To measure the degree of agreement the Cohen's Kappa ( $\kappa$ ) statistic (Cohen, 1960) is often used. The statistic tries to accounts for random agreement: $\kappa$  = (P(agreement) P(e)) / (1-P(e)), where P(agreement) is the fraction of objects which are in the same class for both observers (the main diagonal in the contingency table), and P(e) is the probability of random agreement, given the marginals (like in a Chi-square test). If the observers are in complete agreement the Kappa value  $\kappa$  = 1. If there is no agreement among the observers, other than what would be expected by chance (P(e)), then  $\kappa$  = 0.
- 5.3 The standard Cohen's Kappa statistic only considers perfect agreement versus non-agreement. If one wants to take the degree of disagreement into account (for example with ordinal characteristics), one can apply a linear or quadratic weighted Kappa (Cohen, 1968). If we want to have a single statistic for all observers simultaneously, a generalized Kappa coefficient can be calculated. Most statistical packages, including SPSS, Genstat and R (package Concord), provide tools to calculate the Kappa statistic.
- 5.4 As noted, a low κ-value indicates poor agreement and values close to 1 indicate excellent agreement. Often scores between 0.6-0.8 are considered to indicate substantial agreement, and above 0.8 to indicate almost perfect agreement. If needed, z-scores for kappa (assuming an approximately normal distribution) are available. The criteria for experienced DUS experts could be more stringent than for inexperienced staff.

# 6. Trial design

6.1 If we have multiple observers in a trial, the best approach is to have one person observe one or more complete replications. In that case, the correction for block effects also accounts for the bias between observers. If more than one observer per replication is needed, extra attention should be given to calibration and agreement. In some cases, the use of incomplete block designs (like alpha designs) might be helpful,

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and an observer can be assigned to the sub blocks. In this way we can correct for systematic differences between observers.

# 7. Example of Cohen's Kappa

7.1 In this example, there are three observers and 30 objects (plots or varieties). The characteristic is observed on a scale of 1 to 6. The raw data and their tabulated scores are given in the following tables:

	Ob	Ob	Oh
Variation	Observer	Observer	Observer
Variety	1		3
VI	1	1	1
V2	2	1	2
V3	2	2	2
V4	2	1	2
V1 V2 V3 V4 V5 V6 V7 V8	2 2 2 2 2 2 2 2 3 3 3 4 2 2 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	2 1 1 2 1 1 1 2 1	2
V6	2	1	2
V /	2	2	2
	2	1	2
V9	2	1 1	2
V10	3	1	3
V11	3	1 2 5	3
V12	3	2	2
V13 V14	4	5	4
V14	2	1	1
V15	2	1 2 4 2 1 2	2
V16	2	2	3
V17 V18	5	4	5
V18	2	2	3
V19	1	1	1
V20	2	2	2
V21	2		2
V20 V21 V22	2 1 2 2 1 6	1	1
V23	6	3	6
V24	5	6	6
V25	2	1	2
V23 V24 V25 V26 V27 V28 V29	2 6 2 5	6	3 1 2 2 2 2 2 2 2 2 2 2 2 3 3 2 4 1 2 3 5 3 1 2 2 1 6 6 2 6 2 5 5 4
V27	2	6	2
V28	5	6	5
V29	6	6	5
V30	4	4	4

Scores for						
variety	1	2	<u>3</u>	4	5	6
V1	3 1 0	2 0 2 3 2 2 2 2 3 2 2		0	0	0
V2	1	2	0	0	0	0
V3	0	3	0	0	0	0
V3 V4 V5	1	2	0	0	0	0
V5	1	2	0	0	0	0
V6	1	2	0	0	0	0
V7	0	3	0	0	0	0
V8	1	2	0	0	0	0
V9	1	2	0 2 2 1	0	0	0
V10	1	0	2	0	0	0
V11	1	0	2	0	0	0
V12	0	2 0		0	0	0
V13	0		0	2 0	1	0
V14	2 1	1 2 2 0	0		0	0
V15	1	2	0	0	0	0
V16	0	2	1	0 1 0	0	0
V17	0	0	0	1	2	0
V18	0	2 0	1	0	0	0
V19	3	0	0	0	0	0
V20	0	3 2	0	0	0	0
V21	1	2	0	0	0	0
V22	3	0	0	0	0	0
V23	0	0	1	0	0	2 2 0
V24	0	0	0	0	1	2
V25	1	2 0	0	0	0	0
V26	0		0	0	0	3 1
V27	0	2 0	0	0	0	1
V28	0		0	0	2	1 2
V29	0	0	0	0	1	2
V30	0	0	0	3	0	0

The contingency table for observer 1 and 2 is:

01\02	1	2	3	4	5	6	Total
1	3	0	0	0	0	0	3
2	10	5	0	1	0	1	17
3	2	1	0	0	0	0	3
4	0	0	0	1	0	0	1
5	0	0	0	1	0	2	3
6	0	0	1	0	0	2	3
Total	15	6	1	3	0	5	30

The Kappa coefficient between observer 1 and 2, κ(O1,O2) is calculated as follows:

- $\kappa(O1,O2) = (P(agreement between O1 and O2) P(e)) / (1 P(e)) where:$
- P(agreement) =  $(3+5+0+1+0+2)/30 = 11/30 \approx 0.3667$  (diagonal elements)
- P(e) =  $(3/30).(15/30) + (17/30).(6/30) + (3/30).(1/30) + (1/30).(3/30) + (3/30).(0/30) + (3/30).(5/30) \approx 0.1867$ . (pair-wise margins)
- So  $\kappa(O1,O2) \approx (0.3667-0.1867) / (1-0.1867) \approx 0.22$

This is a low value, indicating very poor agreement between these two observers. There is reason for concern and action should be taken to improve the agreement. Similarly the values for the other pairs can be calculated:  $\kappa(O1,O3) \approx 0.72$ ,  $\kappa(O2,O3) \approx 0.22$ . Observer 1 and 3 are in good agreement. Observer 2 is clearly different from 1 and 3 and the reasons for the deviation requires further investigation (e.g. consider need for additional training).

# TC/52/16 Annex, page 5

# 8. References

**Cohen, J**. (1960) A coefficient of agreement for nominal scales. Educational and Psychological Measurement 20: 37-46.

**Cohen, J.** (1968) Weighted kappa: Nominal scale agreement provision for scaled disagreement or partial credit. Psychological Bulletin, 70(4): 213-220.

**Bland, J. M. Altman D. G.** (1986) Statistical methods for assessing agreement between two methods of clinical measurement, Lancet: 307–310.

http://www.seedtest.org/en/stats-tool-box- content---1--1143.html (ISO 5725-2 based software)

[End of Annex and of document]